UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460 ·

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Tuesday, January 29, 2008

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No.:**84622-R**
Product name:**Microlite**
DP Barcode: D345191

FROM: Earl Goad
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

THRU: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

THRU: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

TO: Emily Mitchell PM#32/Thomas Luminello, Jr.
Regulatory Management Branch II
Antimicrobials Division (7510P

Applicant: BarrierSafe Solutions International
2301 Robb Drive
Reno, NV 89523

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
020502	Sodium Hypochlorite	8.00
	Other Ingredient(s):	92.00
	Total:	100.00

1) BACKGROUND: BarrierSafe Solutions International has submitted a six pack of acute toxicity studies(oral, dermal, inhalation, eye and dermal irritation and dermal sensitization) for the proposed product, "Microlite". This is an end user product containing sodium chlorite as the active ingredient intended for formulation into exempted treated articles.

The Product Science Branch (PSB) /Antimicrobials Division (AD) contractor, Computer Sciences Corporation (CSC), conducted a primary review of the six studies. The Chemistry and Toxicology Team (CTT) conducted a brief secondary review to assure that the studies meet EPA/OPP criteria. Findings and recommendations are the product of this review process.

2) FINDINGS:

a)Each of the six Acute Toxicity Studies is Acceptable.

b) The acute toxicity profile for **84622-R Microlite** is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	472350-03	III	Acceptable
Acute Dermal Toxicity	472350-04	IV	Acceptable
Acute Inhalation Toxicity	472350-05	IV	Acceptable
Primary Eye Irritation	472350-06	II	Acceptable
Primary Skin Irritation	472350-07	IV	Acceptable
Dermal Sensitization	472350-08	Non-Sensitizer	Acceptable

3) LABELING: Corrections to label based on above acute toxicity profile.

a) The signal word is **WARNING**, based on the Primary Eye Irritation being category II.
b) Precautionary Statements:

i) Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear "protective eyewear". Harmful if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

ii) The label submitted states additional Precautionary Statements as follows: "Harmful if inhaled. Do not breathe dust or vapor." These are, acceptable but not required.

c) First Aid Statements:

i) If in eyes:

- Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye..
- Call a poison control center or doctor for treatment advice.

ii) If swallowed:

- Call a poison control center or doctor immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by a poison control center or doctor.
- Do not give anything by mouth to an unconscious person.

iii) The label was submitted with First Aid Statements for Inhalation and Skin.. These statements are **not required** since the inhalation and dermal toxicity are category IV. If the registrant wishes to include please correct to read as the following statements.

If inhaled:

- Move person to fresh air.
- If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
- Call a poison control center or doctor for further treatment advice.

If on skin

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)
(UP AND DOWN PROCEDURE)

Product Manager: 32
MRID No.: 472350-03

Reviewer: CSC and Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21698

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Dosage: Limit Test: 5,000 mg/kg (administered as a 50% w/w mixture in distilled water)
Main Test: 175, 550, 1,750, and 5,000 mg/kg (administered as a 50% w/w mixture in distilled water)

Species: 11 Rats; Sprague-Dawley derived, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (9-10 weeks old)
Weight: 164-202 grams at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 20-24°C
Humidity Range: 30-48%
Photoperiod: 12-hour light/dark cycle
Acclimation: 9-16 days

Conclusion:

1. **Acute Oral LD_{50} (mg/kg)**: Female Rats: 1,750 mg/kg
95% Confidence Interval: 1,239 to 4,450 mg/kg

2. **Toxicity Category**: III **Classification**: Accepted

Procedure (Deviations from 870.1100):

- No protocol deviations were reported.
- Food was resumed 3-4 hours after dosing, fasting period was not reported.
- The guidelines state that animals should be observed individually at least once during the first 30 minutes after dosing. The laboratory stated that the animals were observed during the first several hours post-dosing. Data reported (in Table 2 of the report) identify observations made at 1 hour.
- Individual body weights of test animals were recorded; however, changes in body weights were not calculated.

Results:

Limit and Main Tests

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
Limit Test				
1	3101	5,000	Cyanosis	D
Main Test				
1	3102	175	S	S
2	3103	550	S	S
3	3104	1,750	S	D
4	3105	550	S	S
5	3106	1,750	S	S
6	3107	5,000	D	D
7	3108	1,750	S	S
8	3109	5,000	D	D
9	3110	1,750	S	S
10	3111	5,000	D	D

S – Survival; D – Death

Observations:

175 mg/kg (1 animal) and 550 mg/kg (2 animals) Dose Levels

All animals survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

1,750 mg/kg Dose Level (4 animals)

One animal died within two days of test substance administration. Prior to death, the animal was hypoactive and exhibited cyanosis, piloerection, and reduced fecal volume. The surviving animals exhibited signs of cyanosis, piloerection, and/ or reduced fecal volume. However all animals recovered by Day 2 and appeared active and healthy for the remainder of the study, gaining body weight over the entire 13-day observation period.

5,000 mg/kg Dose Level (4 animals)

All animals died within three hours of test substance administration. Prior to death, all animals were hypoactive and exhibited cyanosis and/or piloerection.

Gross Necropsy Findings:

175 mg/kg (1 animal) and 550 mg/kg (2 animals) Dose Levels

No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

1,750 mg/kg Dose Level (4 animals)

Gross necropsy of the decedent revealed red intestines. No gross abnormalities were found for the euthanized animals when necropsied at the conclusion of the 14-day observation period.

5,000 mg/kg Dose Level (4 animals)

Gross necropsy of the decedents revealed red intestines.

Statistical Analysis:

The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Westat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations, and/or LD_{50} and confidence limit calculations.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 32
MRID No.: 472350-04

Reviewer: CSC and Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21699

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Dosage: 5,000 mg/kg (applied as a dry paste; 75% w/w mixture in distilled water)

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (9-10 weeks old)
Weight: Males: 301-333 grams; Females: 206-235 grams; at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature: 20-23°C
Humidity: 31-39%
Photoperiod: 12-hour light/dark cycle
Acclimation: 15 days

Conclusion:

1) **Acute Dermal LD_{50} (mg/kg)**: Male and Female Rats: >5,000 mg/kg

2) **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1200):

- No protocol deviations were reported.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups simultaneously.
- Individual body weights of test animals were recorded; however, changes in body weights were not calculated.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
5,000	0 / 5	0 / 5	0 / 10

Observations:
All animals survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior.

Gross Necropsy Findings:
No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)

Product Manager: 32
MRID No.: 472350-05

Reviewer: CSC and Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21700

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (9-10 weeks old)
Source: Ace Animals, Inc., Boyertown, PA
Weight: Males: 302-322 grams; Females: 192-238 grams; at experimental start
Housing: Temperature: 20-23°C
Humidity: 47-63%
Photoperiod: 12-hour light/dark cycle
Acclimation: 14 days

Exposure Concentration:

Group	Gravimetric Exposure Concentration (mg/L)[1]	Nominal Concentration (mg/L)
I	2.08	8.65

Conclusion:

1. **LC_{50} (mg/L) 4-hr exposure**: >2.08 mg/L in male and female rats.
2. **The estimated 4-hr acute inhalation LC_{50} of Microlite is greater than 2.08 mg/L in male and female rats.**
3. **Average MMAD**: 3.25 µm
4. **Toxicity Category**: IV **Classification** Acceptable

Procedure (Deviations from 870.1300):

- No protocol deviations were reported.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is exposed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups simultaneously.
- The laboratory report did not identify the mean oxygen content; therefore, it is unknown whether an adequate oxygen content of at least 19 percent was maintained during exposure. However, the guidelines state that it is normally not necessary to measure chamber oxygen concentration if airflow is adequate. The laboratory reported 284 air changes per hour during the study.

- The laboratory does not indicate whether animals were acclimated to exposure conditions and heat stress minimized.
- The guidelines state that three to four measurements should be taken during exposure if chamber concentration values and MMAD values taken during the trial run measurements are not within 10 percent of each other. The laboratory reported four trial runs with chamber concentration values ranging from -2 to 4.30 mg/L. MMAD values were reported for one of the four trial runs. The laboratory conducted only two sample measurements during the test, instead of the three to four measurements recommended in the guidelines.
- The guidelines state that temperature and humidity during exposure should be recorded at least 3 times. The laboratory reported temperature and humidity of the air during exposure as a range, not individually.
- Individual body weights of test animals were recorded; however, changes in body weights were not calculated.
- The guidelines state that the laboratory should report information regarding the treatment of exhaust air. The laboratory report did not describe methods or equipment used to treat exhaust air.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Total
2.08	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	[1]Cumulative % of Particles < Effective Cutoff Diameter (µm)								
				0.0	0.4	0.7	1.1	2.1	3.3	4.7	5.8	9.0
2.08	1	3.6	2.26	0.0	0.3	2.8	12.0	29.3	43.1	52.9	58.4	67.8
	2	3.8	2.50	0.0	0.6	3.4	12.3	29.0	42.6	52.6	59.2	69.3

[1]Percent of particles smaller than corresponding effective cutoff diameter.

Chamber Environment During Exposure

Exposure Level (mg/L)	2.08
Chamber Volume (L)	6.7
Average Total Airflow (Lpm)	31.7
Number of Air Changes Per Hour	284
Mean Oxygen Content (%)	not reported
Temperature Range (°C)	22-22
Relative Humidity Range (%)	50-53

Clinical Observations:
All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. Over the entire 14-day observation period following exposure, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

Gross Necropsy Findings:

No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 32
MRID No.: 472350-06

Reviewer: CSC and Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21701

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Dosage: 0.1 gram (instilled as the powder received) right eye, left eye as control.
Dosing performed after 2 drops of ocular anesthetic (Tetracaine Hydrochloride Opthalmic Solution) was placed in each eye.

Species: 3 Rabbits; New Zealand, albino
Sex: 3 Females. Females were nulliparous and non-pregnant.
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature: 19-22°C
Humidity: 48-61%
Photoperiod: 12-hour light/dark cycle
Acclimation: 12 days

Conclusion:

1. **Toxicity Category**: II (moderately irritating)

2. **Classification**: Acceptable

Procedure (Deviations from 870.2400):

- The laboratory report noted the following protocol deviation: "Due to a technician error, the conjunctivae redness score for Animal No. 3401 on Day 10 was inadvertently not recorded with the rest of the Day 10 scores. The error was discovered on Day 11 and the irritation observed was recorded. The redness score observed on Day 11 was consistent with what was observed on Days 7 and 14. For the purpose of summarizing the incidence of positive effects and the severity of irritation, the Day 11 conjunctivae score will be used for the Day 10 calculation. This deviation had no impact on the outcome of the study."

Results:
All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. One hour after test substance instillation, all three treated sites exhibited iritis and "positive" conjunctivitis. Within 24 hours, two treated eyes exhibited corneal opacity. The overall incidence and severity of irritation decreased gradually thereafter. All animals were free of ocular irritation by Day 17 (study termination).

The Maximum Mean Total Score of the test substance, Microlite, is 21.7. Under the conditions of this study, Microlite is classified as moderately irritating to the eye.

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			Severity – Mean Score
	Corneal Opacity	Iritis	Conjunctivitis	
1 hour	1 / 3	3 / 3	3 / 3	20.0
24 hours	2 / 3	3 / 3	3 / 3	21.7
48 hours	2 / 3	2 / 3	3 / 3	18.0
72 hours	2 / 3	2 / 3	3 / 3	16.7
Day 4	1 / 3	0 / 3	2 / 3	10.3
Day 7	0 / 3	0 / 3	1 / 3	5.3
Day 10	0 / 3	0 / 3	0 / 3	2.0
Day 14	0 / 3	0 / 3	0 / 3	0.7
Day 17	0 / 3	0 / 3	0 / 3	0

Individual Scores for Ocular Irritation

Observations	Rabbit No. 3401 (Female)								
	Hours After Treatment				Days After Treatment				
	1	24	48	72[1]	4	7	10	14	17
I. Corneal Opacity	0	0[2]	0	0	0	0	0	0	0
II. Iris	1	1	0	0	0	0	0	0	0
III. Conjunctivae									
A. Redness	2	2	2	2	2	1	1[3]	1	0
B. Chemosis	2	2	1	1	1	0	0	0	0
C. Discharge	3	2	2	1	1	1	0	0	0
Observations	**Rabbit No. 3402 (Female)**								
	Hours After Treatment				Days After Treatment				
	1	24	48	72	4	7	10	14	17
I. Corneal Opacity	0	1[2]	1[2]	1[2]	0	0	0	0	0
II. Iris	1	1	1	1	0	0	0	0	0
III. Conjunctivae									
A. Redness	2	2	2	2	1	1	0	0	0
B. Chemosis	2	2	1	1	1	0	0	0	0
C. Discharge	2	2	2	2	1	1	0	0	0
Observations	**Rabbit No. 3403 (Female)**								
	Hours After Treatment				Days After Treatment				
	1	24	48	72	4	7	10	14	17
I. Corneal Opacity	1	1[2]	1[2]	1[2]	1	0[2]	0	0	0
II. Iris	1	1	1	1	0	0	0	0	0
III. Conjunctivae									
A. Redness	2	3	3	2	2	2	1	0	0
B. Chemosis	2	2	2	2	2	1	0	0	0
C. Discharge	3	3	2	2	2	1	1	0	0

[1]There appeared to be residual test substance in the eye. As a result of this, the eye was flushed with saline. However, after the saline flush the test material still remained present in the eye.
[2]2% ophthalmic fluorescein sodium used to evaluate the extent or verify the absence of corneal opacity.
[3]Day 10 scoring for redness was performed on Day 11 (see protocol deviation described above).

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 32
MRID No.: 472350-07

Reviewer: CSC and Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21702

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Dosage: 0.5 gram (applied as a dry paste; 75% w/w mixture in distilled water)

Species: 3 Rabbits; New Zealand, albino
Sex: 3 Male
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature: 21-22°C
Humidity: 49-62%
Photoperiod: 12-hour light/dark cycle
Acclimation: 9 days

Conclusion:

1. **Toxicity Category**: IV (slightly irritating)

2. **Classification**: Acceptable

Procedure (Deviations from 870.2500):

- No protocol deviations were reported or identified.

Results:
All animals appeared active and healthy during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No edema was observed at any treated site during this study. Within one hour after patch removal, all three treated sites exhibited very slight erythema. The overall incidence and severity of irritation decreased with time. All animals were free of dermal irritation by 72 hours.

The Primary Dermal Irritation Index for the test substance, Microlite, was calculated to be 0.5. Under the conditions of this study, Microlite is classified as slightly irritating to the skin.

Incidence of Irritation

Time After Patch Removal	No. of Animals Testing "Positive" / No. of Animals Tested		Severity of Irritation– Mean Score
	Erythema	Edema	
30-60 minutes	3 / 3	0 / 3	1.0
24 hours	2 / 3	0 / 3	0.7
48 hours	1 / 3	0 / 3	0.3
72 hours	0 / 3	0 / 3	0

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema			
		Time After Patch Removal			
		30-60 min	24 hrs	48 hrs	72 hrs
3501	M	1 / 0	1 / 0	1 / 0	0 / 0
3502	M	1 / 0	0 / 0	0 / 0	0 / 0
3503	M	1 / 0	1 / 0	0 / 0	0 / 0
Total		3 / 0	2 / 0	1 / 0	0 / 0
Mean		1.0 / 0	0.7 / 0	0.3 / 0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	Hours			
	30-60 min	24 hrs	48 hrs	72 hrs
Erythema	1.0	0.7	0.3	0
Edema	0	0	0	0
TOTAL (PDI)[2]	1.0	0.7	0.3	0

[1]Average values for three rabbits.
[2]PDI = Average Erythema + Average Edema

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 32
MRID No.: 472350-08

Reviewer::CSCand Earl G. Goad (CTT)
Completion Date: July 9, 2007
Report No.: 21703

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), with the following exception: "The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Eurofins | Product Safety Laboratories historical positive control study were not determined."

Test Material: Microlite
Batch #: Formula NML-001-F / White powder

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)
(Historical data - completed October 11, 2006)

Species: 38 Guinea pigs; Hartley, albino
Sex: Range-Finding: 8 Males
Test Group: 20 Males
Naïve Control Group: 10 Males
Age: Young adult (specific age not reported)
Weight: Test and Naïve Control Groups: 302-422 grams at experimental start
Source: Elm Hill Breeding Labs, Chelmsford, MA
Housing: Temperature: 19-23°C
Humidity: 45-62%
Photoperiod: 12-hour light/dark cycle
Acclimation: 6-15 days

Method: Buehler Method

Conclusion:

Based on these findings and on the evaluation system used:

1) **Microlite is not considered to be a contact sensitizer.**

2) **Classification**: Acceptable

Procedure (Deviations from 870.2600):

- No protocol deviations were reported.
- The laboratory only graded erythema, and not edema, although the guidelines require that as a minimum, the erythema and edema must be graded.

Procedure:

Preliminary Irritation Testing: A group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was mixed with a solution of 2% carboxymethyl cellulose in distilled water to yield w/w concentrations of 75%, 56%, 38%, 19%, 12%, 6%, 3%, and 1%. Each concentration was applied (0.4 gram each) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. The HNIC selected for the challenge phase was a 3% w/w mixture in a solution of 2% carboxymethyl cellulose in distilled water.

Preparation and Selection of Animals: Prior to initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, four-tenths of a gram of a 38% w/w mixture of the test substance in a solution of 2% carboxymethyl cellulose in distilled water was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty-seven days after the first induction dose, four-tenths of a milliliter of a 3% w/w mixture of the test substance in a 2% solution of carboxymethyl cellulose in distilled water (HNIC) was applied to a naïve site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, PSL Study #20608, was performed by Eurofins | Product Safety Laboratories. Testing was completed on October 11, 2006. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described above.

Results:

Induction Phase:
Test Animals (38% w/w mixture of the test substance in a solution of 2% carboxymethyl cellulose in distilled water): Faint to moderate erythema (1-2) was noted for all test sites during the induction phase.

Historical Positive Control Animals (HCA applied undiluted): Very faint to faint erythema (0.5-1) was noted for all positive control sites during the induction phase.

Challenge Phase:
Test Animals (3% w/w mixture of the test substance in a solution of 2% carboxymethyl cellulose in distilled water): Very faint erythema (0.5) was noted at thirteen of twenty test sites 24 hours after challenge. Similar irritation persisted at ten sites through 48 hours.

Naïve Control Animals (3% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted at four of ten naïve control sites 24 hours after challenge. Similar irritation persisted at three sites through 48 hours.

Historical Positive Control Animals (75% w/w mixture of HCA in mineral oil): Six of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar irritation persisted at three sites through 48 hours. Very faint erythema (0.5) was noted for all other sites after challenge.

Historical Naïve Control Animals (75% w/w mixture of HCA in mineral oil): Very faint erythema (0.5) was noted for two of five naïve control sites 24 hours after challenge. Irritation persisted at one of these sites through 48 hours.

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		**Severity[2]**	
	Hours		**Hours**	
	24	**48**	**24**	**48**
Test Animals – Challenge	0 / 20	0 / 20	0.33	0.25
Naïve Control Animals – Challenge	0 / 10	0 / 10	0.2	0.15

[1]Animals with scores greater than 0.5.
[2]Sum of the erythema scores divided by the number of animals evaluated.

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge	
	1		2		3			
Concentration[1]	38%		38%		38%		3%	
Hours[2]	24	48	24	48	24	48	24	48
Animal No. / Sex								
Test Group								
3601 / M	2	2	2	2	2	2	0.5	0
3602 / M	2	2	2	1	2	1	0	0
3603 / M	1	1	1	1	1	1	0.5	0
3604 / M	2	2	2	2	2	2	0.5	0.5
3605 / M	1	1	2	1	2	1	0.5	0.5
3606 / M	2	2	2	2	1	1	0.5	0.5
3607 / M	2	1	1	1	1	1	0.5	0.5
3608 / M	1	1	2	1	1	1	0	0
3609 / M	1	1	2	2	2	2	0	0
3610 / M	2	2	1	1	2	1	0.5	0.5
3611 / M	2	2	2	2	2	2	0.5	0.5
3612 / M	2	2	2	2	2	2	0.5	0.5
3613 / M	1	1	2	1	2	1	0.5	0.5
3614 / M	2	2	2	2	2	1	0	0
3615 / M	1	1	2	1	1	1	0	0
3616 / M	2	2	2	2	2	2	0	0
3617 / M	2	1	1	1	1	1	0.5	0
3618 / M	2	1	1	1	1	1	0.5	0.5
3619 / M	2	1	2	2	2	2	0.5	0.5
3620 / M	1	1	1	1	1	1	0	0
Naïve Control Group								
3621 / M	--	--	--	--	--	--	0.5	0
3622 / M	--	--	--	--	--	--	0.5	0.5
3623 / M	--	--	--	--	--	--	0	0
3624 / M	--	--	--	--	--	--	0.5	0.5
3625 / M	--	--	--	--	--	--	0	0
3626 / M	--	--	--	--	--	--	0	0
3627 / M	--	--	--	--	--	--	0	0
3628 / M	--	--	--	--	--	--	0	0
3629 / M	--	--	--	--	--	--	0.5	0.5
3630 / M	--	--	--	--	--	--	0	0

[1]The test substance (induction phases) was applied as a 38% w/w mixture in a solution of 2% carboxymethyl cellulose in distilled water. The test substance (challenge phase) was applied as a 3% w/w mixture in a solution of 2% carboxymethyl cellulose in distilled water.
[2]Hours after induction dose.